

## COMPARATIVE SURFACE ARCHITECTURAL STUDY OF PAIRED FINS OF HILL- STREAM FISHES *BOTIA ALMORHAE*, *HOMALOPTERA BRUCEI* AND *SCHIZOTHORAX RICHARDSONII*: SEM INVESTIGATION

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### ABSTRACT

Fins are generally used by fishes to achieve all forms of locomotion, stabilization, balancing, change of direction and brake in the aquatic environment. The epidermis is composed mainly epithelial cells, mucous cell and uncular region. The free surface of epithelial cells are possesses microridges, microridges consisting varied patterns at different location in different fish species. These microridges have been correlated to provide reserve surface area for stretching, when manoeuvring of fish. We describe the adhesive nature of the paired fin (pectoral and pelvic) in the *Botia almorhae*, *Homaloptera brucei* and *Schizothorax richardsonii* as examined by scanning electron microscopy, Many Himalayan fishes demonstrate several unique adaptive modifications.

**KEYWORDS:** Hill Stream Fish, Modification, Paired Fins and SEM

### INTRODUCTION

The paired fins are horizontally placed and their outer rays are greatly flattened. This change is brought about for two reasons: first, to allow the ventral surface to be firmly applied to rocks and second, to enable the fins to act as organs of adhesion. *H. brucei* have large pectoral fins that they use as aerofoils to "wing" themselves down onto a rock in the water flow. They utilize the passing water and by angling the fins they create reverse lift and "fly" downwards. Without the sucker adaptations, their ability to maintain station in a really fast flow is reduced. Fins give fish mobility, stability and maneuverability. Paired fins permit the fish to steer, stabilize and stop. Paired fins that are maneuverable and flexible provide better control at slow speeds, whereas fins that are rigid act as steering planes for fast swimming fish.

Fish skin differs from other exposed vertebrate skin most notably at the surface where living epidermal cells are in direct contact with the environment. The epidermis has a fundamental role in the regenerative process of fish fins (Becerra et al., 1983; Akimenko et al., 1995), since the healing time of this structure leads to a faster process, avoiding specially, infective biotic processes by microorganisms found in water environments, such as fungus, bacteria and protozoan that can transmit diseases. The objective of this study was to compare the surface architecture of the fin epidermis of *B. almorhae*, *H. brucei* and *S. richardsonii* using scanning electron microscopy.

### MATERIAL AND METHODS

Live adult specimen of *B. almorhae* (Teleostei: Cobitidae), (Approximately 4-5 inches in length) shall be collected from river Kosi at Kakrighat Distt. Nainital (elevation- 1200m above mean sea level), *H. brucei* (Teleostei: Balitoridae), (Approximately 2-3 inches in length) shall be collected from west Ramganga at Chaukhutia Distt. Almora (elevation-1200m above mean sea level) and *S. richardsonii* (Teleostei: Cyprinidae), (Approximately 4-5 inches in length) shall be collected from river Kosi at Hawalbagh Distt. Almora (elevation-1194 m above sea level) Uttarakhand.

The water current is very fast having the velocity 0.5 to 2.0 m/sec. (Bhatt and Pathak, 1991) and the bed is rocky. To study the details of the morphological adaptations in the fishes, SEM was done. The following procedure was adopted for the preparation of specimen for SEM.

Specimen was maintained in laboratory at  $25 \pm 2^{\circ}\text{C}$ . The fishes were cold anesthetized following Mittal and Whitear (1978), for SEM preparation of fin. Section were cut of the fins with the help of sharp blade and rinsed in 70% ethanol and one change saline solution to remove debris and fixed in 3% Glutaraldehyde in 0.1M phosphate buffer at pH 7.4 overnight at  $4^{\circ}\text{C}$  at refrigerator. The tissues were washed 2-3 changes in phosphate buffer and dehydrated in ascending series of ice cold Acetone (30%, 50%, 70%, 90% and 100% approximate 20-30min.) and critical point dried, using critical point dryer (BIO-RAD England) with liquid carbon dioxide as the transitional fluid. Tissues were glued to stubs, using conductive silver preparation (Eltecks, Corporation, India). The samples were coated with gold using a sputter coater (JFC 1600), examined under (JEOL, JSM- 6610 LV) scanning electron microscope and the images were observed on the screen.

## RESULTS

The epidermis covering of paired fin of these fishes is rough (ray region) and smooth (inter ray region). Rough epidermis also known as keratinize or non-glandular region, that possesses uncini, on the other hand smooth epidermis known as non-keratinize or glandular region, that possesses epithelial cells characterized by different patterns of microridges and mucous cells. In *B. almorhae*, *H. brucei* and *S. richardsonii* the epidermis of ventral surface is much thick at ray region than that of the interrays region (Figure 1, 2 and 3). The ray region of fin provided a large number of horny projections (Uncini) at the surface and so is designated as uncinar epidermis whereas epidermis of rest of the parts of fins is devoid of such projections, hence is designated as smooth epidermis. The epidermal cells of the ray region are thrown into numerous spines like structures, the uncini.

In *H. brucei*, the uncini are numerous, long, curved and backwardly directed in the ray region of pectoral fin (Figure 4). On the other hand the cells that line the interray region are apparently devoid of these uncini. In pelvic fins of *H. brucei*, the uncini of the ray region are stout, that often have slightly curved apical ends could serve as pegs or anchors (Figure 5), as well as very thick, stumpy and backwardly directed in the ray region of pectoral fin of *B. almorhae* (Figure 6), this type structures are not found in the *S. richardsonii*.

In *B. almorhae*, *H. brucei* and *S. richardsonii* the smooth epidermis, epithelial cells are polygonal, characterized by microridges, polygonal epithelial cells of varied dimensions (Figure 7, 8 and 9). Surface epithelial cells in *B. almorhae* are modified into epidermal outgrowth, the uncini. These uncini are short and stumpy structures. The tiny projection arising out of the epithelial cells, the base of uncini of *Botia* possesses polygonal epithelial cells indicating that these uncini are the modification of epithelial cells in the fish (Figure 7), but absent in the epithelial cells of *H. brucei* and *S. richardsonii*. On the dorsal and ventral surface the smooth epidermis is present in both the fins, epithelial cells are characterized with microridges, and the microridges on the epithelial cells are also of interesting that periodically they seem to be expanded in the form of a villus-like structure (figure 8) in *H. brucei*. In case of *S. richardsonii* the epithelial cells of paired fin display a labyrinthine pattern (Figure 9) of microridges with a more or less continuous circumferential ridge demarcating the boundaries of the polygonal cells. Interspersed between the epithelial cells, mucous cells apparatus are distinguished, the openings of mucous cells were visible among the junctions of the epithelial cells (Figure 9).

It is interesting to note that a large number of well protruded tubercles appearing to be cleaved in the centre are present in between the epithelial cells of *H. brucei* (Figure 10). The density of these tubercles is, however, much greater in

dorsal surface than that of ventral surface. Roberts (1982) also described such tubercles in a large number of Ostariophysean fishes. They are well developed in the dorsal surface of the paired fins in sexually mature males and poorly developed or absent in females, this type structures are not found in *B. almorhae* and *S. richardsonii*.

## DISCUSSIONS

Surface architecture of paired fins of *B. almorhae*, *H. brucei* and *S. richardsonii* adapted to life in fast flowing streams has been examined. Epidermis is differentiated into smooth and rough in both the fins. In smooth epidermis show the epithelial cells, the epithelial cells showed numerous microridges, these microridges are, different patterns in different fishes. The epithelial cells are demarcated by well marked boundaries. Fishelson, (1984) correlated the variations in micro-ridge pattern with the locomotory activity of the fish. Fishelson suggested that in faster swimming fishes, micro-ridges are most developed and serve to trap mucus on the epithelial surface.

Rough epidermis covering ventral surface is composed of mainly epithelial cells which are thrown into elongated curved uncini in pectoral fin and stout and blunt uncini in pelvic fin on other hand, uncini are stout in both the fins *B. almorhae*. The well-developed unciniferous pads on the ventral surface of paired fins, in addition to providing adhesion, may help achieve the seal for the suction device, possibly the seal enhanced by suction or seizing due to exit of water from the interradial grooves, between unciniferous pads. The presence of uncini and differences in these structures of different fish could be considered as adaptive modifications that reflect varied functional demands. When the fishes are pressed against the substratum; a reduced pressure is created by the musculature attached to the ridges and grooves. The spiny projections might then assist in organic growth on the submerge rocks (Joshi et al. 2010). Hard unicellular projections of varied shape arising from epidermal cells- are an adaptive feature of major importance to balitorid loaches. The surface of the velvety pads on the ventral surface of the expanded paired fins consists solely of thousands of uncini. The unciniferous pads evidently serve as friction pads. Without uncini, such expanded paired fins presumably would not have evolved, nor would have the rows or patches of tubercular contact organs on the dorsal surface of the pectoral fins (Tyson and Roberts, 1997).

The mucus secretion from the mucous glands causes a weak adhesion and prepares the sub-stratum for subsequent action of the spines. In addition, the mucus seems to afford protection to the spines from abrasion during adhesion. The apparent lack of spines in some of the epidermal cells (located near the base of the ridges) indicates that these structures are often damaged and then possibly lost, to generate new spines. The factors causing damage to the spines could be the constant mechanical abrasion of reduced mucus secretion from the surrounding mucous glands in altered physiological states (Joshi, et al., 2011). Fishes inhabiting hill streams require special modifications in their body forms to prevent themselves from being washed away by the fastly flowing current of water. Such modifications are chiefly manifested in the form of adhesive structure usually located on the ventral side of the body at the anterior end and on the fin (Sinha, et al., 1990).

The integument of the majority of the teleost is characterized by the presence of numerous epidermal mucous cells (Henrikson and Matoltsy 1968; Whittar 1986; Abraham et al. 2001). Because the pectoral and pelvic fins are covered with epidermis, it is not surprising to find mucous glands there in. However, it is difficult to suggest why glandular openings are absent in the contour area of the outer rays of the pelvic fins.

Fish mucus covers every external surface of a fish: its skin, gills, and eyes, as well as the inner surface of its digestive tract. As the outermost surface of a fish, the epithelial mucus layer mediates most of a fish's interaction with its environment. The functions of epidermal mucus are numerous -Reduction of turbulence (rheology), regulation of chemical

exchange with surrounding water, protection from ultraviolet radiation, provision of nutrition for other organisms, predator/Prey interactions and antimicrobial activity.

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## REFERENCES

1. Abraham, M., Iger, Y. and Zhang, L. (2001). Fine structure of the skin cells of a stenohaline freshwater fish *Cyprinus carpio* exposed to diluted seawater. *Tissue Cell* 33, 46-54.
2. Akimenko, M. A., Johnson, S. L., Westerfield, M. and Ekker, M. (1995). Differential induction of four msx homeobox genes during fin development and regeneration in zebrafish. *Development*, vol. 121(2), 347-357.
3. Becerra, I. J., Montes, G. S., Bexiga, S. R.R. and Junqueira, L.C.U. (1983). Structure of the tail fin in teleosts. *Cell and Tissue Research*, vol. 230(1), 127-137.
4. Bhatt, S. D. and Pathak, J. K. (1991). Streams of Great Mountain are: Physiography and Physiochemistry. In: Ecology of the mountain water. *Ashish publ.* House N. Delhi., pp. 43-58.
5. Fishelson, L. (1984). A comparative study of ridges-mazes on surface epithelial cell-membranes of fish scales (Pisces, Teleostei). *Zoomorphologie*. 104, 231-238.
6. Henrikson, R. C. and Matoltsy, A. G. (1968). The fine structure of teleost epidermis. II. Mucus cells. *J. Ultrastruct. Res.* 21, 213-221.
7. Joshi, S. C., Bisht, I. and Agarwal, S. K. (2010). *Adaptive modifications in the epidermis of certain Hill-stream fishes of Kumaun region: A scanning electron microscope investigation*. PhD Thesis, KU. Nainital.
8. Joshi, S. C., Singh, H., Bisht, I. and Agarwal, S. K. (2011). Scanning Electron Microscopic Investigation of "Adhesive Apparatus Epidermis" of *Glyptothorax pectinopterus* (McClelland) (Sisoridae). *Journal of American Science*. 7 (12), 275-280.
9. Mittal, A. K. and Whitear, M. A. (1978). Notes on cold anaesthesia of poikilotherms; *J. Fish. Biol.* 13, 519-520.
10. Sinha, A. K., Singh, I. and Singh, B. R. (1990). The morphology of the adhesive organ of the Sisorid fish, *Glyptothorax pectinopterus*. *Jap. J. of Ichth.* 36, (4), 427-431.
11. Tyson, R. and Roberts, (1997). Systematic reservation of the Balitorid loach genus *Sewellia* of Vietnam and Laos, with diagnosis of four new species. *The raffles bulletin of zoology*. 46(2), 271-288.
12. Whitear, M. (1986). The skin of fishes including cyclostomes. 8-38. Bereiter-Hahn J., Matoltsy G. A. and Sylvia-Richards K. (Ed.). Biology of the integument, Vol.2, *Vetebrates, Spinger-Verlag, Heidelberg*.

## APPENDICES

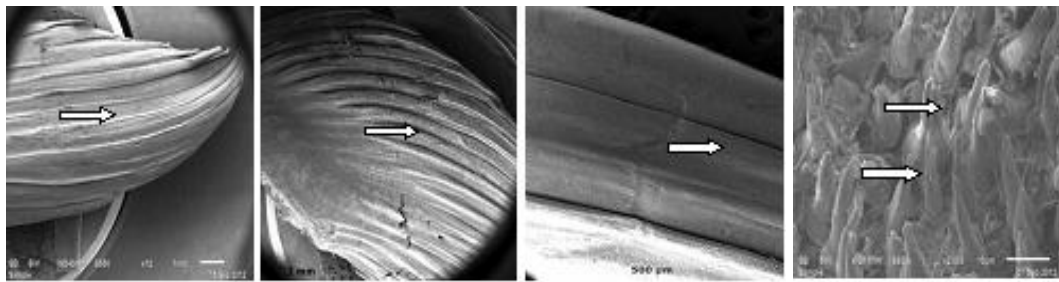


Figure 1

Figure 2

Figure 3

Figure 4

Figures 1, 2 and 3: Surface Electron Microphotograph (SEMPH) of the Paired Fin Epidermis of *B. almorhae*, *H. brucei* and *S. richardsonii* Showing Well Developed Fin Rays (Marked by Arrow) (Scale Bar-500µm, 2mm and 500µm)

Figure 4: SEMPH of Pectoral Fin of *H. brucei* Showing Long Curved Unculi (Scale Bar-10µm)

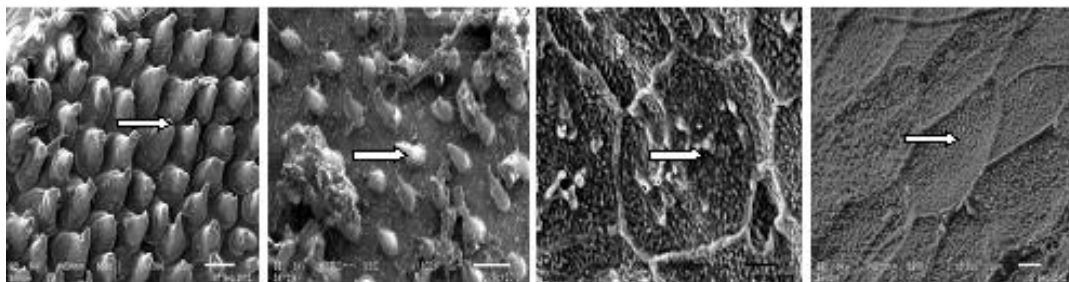


Figure 5

Figure 6

Figure 7

Figure 8

Figure 5: SEMPH of Pelvic Fin of *H. brucei* Showing Unculi at the Ray Region (Scale Bar-10µm)

Figure 6: SEMPH of Pectoral Fin Epidermis of *B. almorhae* Showing Blunt Type of Unculi on the Surface of Ray Region (Marked by Arrows) (Scale Bar- 2µm)

Figure 7: SEMPH of the Paired Fin Epidermis of *B. almorhae* Showing Polygonal Epithelial Cells (Marked by Arrow) (Scale Bar-2µm) and Characterized by Tiny Projection Arising Out of the Epithelial Cells (Marked by Arrow) (Scale Bar-2µm)

Figure 8: SEMPH of the Paired Fin Epidermis of *H. brucei* Showing Polygonal Epithelial Cells Characterized by well Developed Villus-Like Microridges (Marked by Arrow) (Scale Bar-10µm)

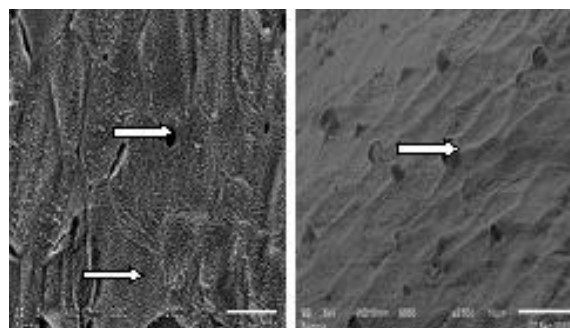


Figure 9

Figure 10

Figure 9: SEMPH of the Paired Fin Epidermis of *S. richardsonii* Showing Polygonal Epithelial Cells, Characterized by Labyrinthine Pattern Microridges (Marked by Arrows) and Showing the Opening of Mucous Cells (Marked by Arrows) (Scale Bar-10µm)

Figure 10: SEMPH of Dorsal Surface of Pectoral Fins of *H. brucei* Epidermis Showing Well Protruded Tubercles in between Epithelial Cells (Scale Bar-10µm)

